Long-term decline in bat activity using passive acoustic monitoring and an equipment correction factor in Nova Scotia, Canada

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Passive acoustic monitoring has grown in popularity as a technique to assess changes in activity levels of various taxa. However, there are few long-term and large-scale acoustic monitoring programs due to the current challenges associated with advancing technology, data management and analyses. The variation in the quality and quantity of acoustic data collected by different equipment setups has become challenging to avoid. There are an increasing number of equipment options that provide different or improved detection capabilities as old models wear and phase out. To assess long-term activity trends of bats between two data sets collected by different equipment in southwest Nova Scotia, Canada, I developed equipment variation correction factors. I compared the assumed proportion of successful detections as given by binomial distribution between two types of bat acoustic monitors positioned side by side. I found the proportion of successful Anabat SD1 to Song Meter SM4 detections to vary by night (n=5), height (3 m, 6 m) and species (*Myotis lucifugus*, *Perimyotis subflavus*). There was no systematic bias in the correction factors when I compared the Anabat to the corrected Song Meter detections as indicated by mean errors centered around zero. After applying the correction factors, acoustic activity of *Myotis lucifugus* declined by 95.50% 95% CI (96.96%, 93.59%) and *Perimyotis subflavus* declined by 91.37% 95% CI (92.99%, 89.49%) between 2005/2006 and 2018/2019 across southwest Nova Scotia. These trends reflect declines in winter colony counts and summer capture rates across eastern North America attributable to the disease White Nose Syndrome (WNS). My results demonstrate that direct comparisons of data sets collected by different acoustic equipment cannot be made and that equipment variation needs to be accounted for in order to assess long-term activity patterns. Exploring techniques to account for equipment variation and their efficacy will increase our ability to use acoustic data to track long-term population trends and manage wildlife populations. Managers can continue to use acoustics to assess population trends in areas with no known hibernation sites, for species difficult to study in hibernacula and to identify areas that may be significant for WNS recovery. In Nova Scotia, Kejimkujik National Park and National Historic Site may serve as an important area for WNS recovery. Periodic monitoring should continue to document population trends and this long-term data set may be used to track summer population changes.

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List of Abbreviations

1. KNPNHS – Kejimkujik National Park and National Historic Site
2. PAM – Passive Acoustic Monitoring
3. *Pd – Pseudogymnoascus destructans*
4. SWNS – southwest Nova Scotia
5. WNS – White Nose Syndrome

Chapter 1
Introduction to Population Monitoring of Bats

1.1 Long-term Population Monitoring

Population monitoring is central to conservation biology to detect population changes, inform management decisions and evaluate the effectiveness of management (Marsh and Trenham 2008). Monitoring programs can improve our ability to collect long-term and reliable population data (Gibbs et al. 1999; Minton 2003). One of the longest running monitoring programs is the North American Breeding Bird Survey (BBS) which serves as the premier source of bird population data (Sauer et al. 2017). The BBS has illustrated the potential of large scale and long-term monitoring programs for other taxa such as bats (North American Bat Monitoring program; NABat) and amphibians (North American Amphibian Monitoring Program; NAAMP; Hudson et al., 2017; Weir et al., 2014; Loeb et al., 2015). These monitoring programs were initiated due to growing concerns of population decline as a result of emerging threats. For birds, the threat of pesticide use in North America led to the development of the BBS (Sauer et al. 2017) while for bats and amphibians, fungal diseases have caused dramatic population declines (Skerratt et al. 2007; Turner et al. 2011). Large scale monitoring programs like the BBS, NABat and NAAMP are generally the most effective means of assessing long-term population trends in populations over large geographic scales (Stem et al., 2005).

Wildlife managers often use multiple techniques in monitoring programs to qualitatively and quantitatively characterize populations. Typically, monitoring is being carried out to study presence-absence, density, or abundance (Marsh and Trenham 2008). Such metrics can be quantified using either invasive or non-invasive techniques such as captures, radio-tracking and visual, auditory or acoustic surveys (Arizaga et al. 2011; Brookes et al. 2013; Reiczigel et al. 2015). Of these techniques, the use of passive acoustic monitors (PAM) for acoustic surveys have become increasingly popular as equipment became relatively affordable and portable (Browning et al. 2017). PAM equipment options today are also typically programmable and have a longer battery life which allows long-term data collection of multiple sites and habitat types simultaneously (Heinicke et al. 2015; Campos-Cerqueira and Aide 2016). Once collected, acoustic data provides an archive of recordings enabling staff with limited expertise to deploy units that can later be reviewed by experts. With these advancements, PAM has been integrated into some multi-method monitoring programs e.g., NABat (Rodhouse et al., 2019).

Acoustic monitoring has been used to study multiple taxa including terrestrial and marine species (Munger et al. 2008; Kalan et al. 2016) and is particularly useful for detecting cryptic species (Williams et al. 2018). Monitoring sounds produced by wildlife may permit detection, identification and localization of species which can provide insight into behaviors and spatial and temporal variation in them (Measey et al. 2017; Charchuk and Bayne 2018). For long-term monitoring, acoustic data may be used to characterize activity patterns, abundance or density of either a single species, taxonomic group or an entire acoustic community (Celis-Murillo et al. 2009; Stevenson et al. 2015; Xie et al. 2017). Acoustic data is most often used to study temporal variation in activity patterns of populations and communities (Sugai et al. 2019). To be able to confidently estimate activity changes, PAM methods must be consistent during successive monitoring periods between sites and years to prevent variation between data sets (Browning et al. 2017). Changes in activity rather than abundance are frequently used as a proxy for population changes as individuals often cannot be identified from acoustic data alone. Depending on the taxa and species, abundance may be estimated in the case where individuals have unique vocal signatures (Salmi et al. 2014; Petrusková et al. 2016) or multiple microphones are used to localize individuals (Blumstein et al. 2011).

1.2 Bat Monitoring

The desire for population monitoring of bats has increased with emerging threats and their role in healthy ecosystems. Bats in general are important worldwide as they support and promote healthy and diverse ecosystems (Kunz et al. 2011) through pollination (Molina-Freaner and Eguiarte 2003), seed dispersion (De Carvalho-Ricardo et al. 2014) and consumption of large quantities of insects (Boyles et al. 2011). Bats may also serve as a biological indicator as they are one of the most diverse groups of vertebrates and are sensitive to many anthropogenic impacts (Jones et al. 2009). The status of many bat species is threatened by habitat degradation and fragmentation, wind energy development, but more recently the introduced fungus, *Pseudogymnoascus destructans* (*Pd*), causing the disease White Nose Syndrome (WNS) which has resulted in dramatic population declines across North America (COSEWIC 2013). WNS has been confirmed in seven Canadian provinces and 35 U.S. states as of August 30, 2019 (U.S. Fish and Wildlife Service 2020) and has killed over six million bats in the first six years after it was discovered (Blehert et al. 2009; Froschauer and Coleman 2012). The fungus causes physiological and energetic imbalances that increase the frequency of arousal during hibernation causing premature depletion of fat reserves that ultimately leads to death (Frank et al. 2019; Haase et al. 2019; Fuller et al. 2020). However, the impact of WNS on bats in North America differs by species. For example, some hibernating species appear to develop tolerance and resistance (e.g., *Myotis lucifugus, Perimyotis subflavus)* while others show no defense mechanism (e.g., *M. septentrionalis;* Frick et al., 2017).

Techniques that are available for assessing population changes of bats include winter colony counts, maternity roost counts, capture rates, and acoustic monitoring (Murray and Kurta 2004; Frick et al. 2010b; Reusch et al. 2019). The assessment of long-term population changes attributable to WNS have primarily relied on winter surveys using colony counts. For example, hibernation counts of bats across eastern North America had declined by an average of 73% in the U.S. up to 2010 (Frick et al. 2010a) and up to 94% in Canada by 2013 (COSEWIC 2013). The urgency for monitoring data due to the rapid decline and ease of colony counts at overwintering sites, has led to comparatively fewer assessments of population changes using other techniques (e.g., capture rates, acoustic activity) as well as monitoring beyond hibernation season. Winter surveys can provide data on a large number of individuals within one area (Weller et al. 2018) whereas individuals are typically more spread out across summer habitat and require more survey effort (Drake et al. 2020). However, monitoring outside of the hibernation season is essential to understand how the impacts of threats such as *Pd* translate into breeding populations. Monitoring known hibernation sites likely only represents a proportion of the population, given it can be difficult to identify these sites (Weller et al. 2018), so it is important to study other habitats and particularly for regions with no known overwintering sites. Specifically, data collected during the summer can be informative of the change in demographic rates such as survival, recovery and fecundity (Frick et al. 2010b).

Studies using summer monitoring to evaluate long-term changes attributable to WNS are limited and only cover a small percentage of the bat community where *Pd* has spread. In the eastern United States, capture rates have declined by up to 99% (Francl et al. 2012; Pettit and O'Keefe 2017; O'Keefe et al. 2019) and similarly there has been significant declines in acoustic activity (Brooks 2011; Dzal et al. 2011; Ford et al. 2011; Nocera et al. 2019a). As for Canada, there has also been a reliance on winter surveys to model WNS-related population crashes (COSEWIC 2013). Using winter survey data from Ontario eastward, *M. lucifugus* and *M. septentrionalis* combined have declined by 94% and *P. subflavus* by over 75% (COSEWIC 2013). Surveys of a handful of pre-WNS to post-WNS *M. lucifugus* maternity colonies in Ontario and Quebec have declined by up to 99% (COSEWIC 2013). Canada represents an important area for the study of *Pd* as a significant portion of the range of WNS-affected species lie here i.e., 50% of the range of *M. lucifugus*, 40% of *M. septentrionalis* and 10% of *P. subflavus* (van Zyll de Jong 1985).

Studies of bats have benefited from the use of acoustic monitors because they are elusive, nocturnal, difficult to catch and emit ultrasonic sounds above the human hearing range. Acoustic data is a valuable component of monitoring efforts (Barclay 1999) as it can provide a more complete inventory of species' since recorders may be able to detect species that are difficult to catch with netting and harp traps (O'Farrell and Gannon 1999). Long-term acoustic monitoring programs for bats include NABat, the UK's National Bat Monitoring Programme and the Indicator Bats program (iBats; Barlow et al., 2015; K. E. Jones et al., 2013). Researchers rely on echolocation calls emitted for navigation, foraging, and socializing (Surlykke and Kalko 2008; Springall et al. 2019; Cortes and Gillam 2020) to identify species presence or absence, distribution, behavior and colony size in some specifics conditions (Faure et al. 1993; Kloepper et al. 2016; Layng et al. 2019). Most often acoustic data is used to assess activity changes as the magnitude of activity between monitoring periods can be used as a proxy for population trends (Sugai et al. 2019). For bats, individuals cannot be reliably identified from acoustic data alone or without the use of a microphone array to spatially distinguish individuals (Barclay 1999; O'Farrell et al. 1999). Regardless, other monitoring techniques may be used in combination with acoustic monitoring to estimate abundance (Flaquer et al. 2007; Weller et al. 2014).

The quantity and quality of acoustic data has been shown to vary with equipment setup. Specifically, the detectability of a frequency at a distance will vary by detector model where some brands and newer models outperform others. Early studies compared the performance of narrow-band detectors (Downes 1982; Waters and Walsh 1994; Forbes and Newhook 2009), then a shift to broad-band detectors allowed detection of vocalizations across a wider frequency range (Parsons 1996). Comparing different brands of PAM setups, Adams et al. (2012), found the Anabat SD2 (Titley

Scientific) detected the fewest signals out of five commonly used brands and none at 85 or 115 kHz. While most research assesses variation related to a component of the monitoring system, e.g., the microphone type, height (Britzke et al. 2010; Loeb et al. 2020), more information is needed to address the differences between different brands and complete setups (Adams et al. 2012). PAM is rapidly advancing as equipment is replaced by newer models with improved or different detection capabilities. Improvement means that comparing results of repeated sampling using the same equipment and methodology is becoming more challenging. However, continuing to use old equipment or methods between successive sampling periods also does not take advantage of new technology or provide a solution when older equipment is discontinued or malfunctions (Rempel et al. 2013). Overall, PAM can help ease the effort required for summer compared to winter bat monitoring, but the current long-term equipment challenges associated with PAM need further exploration.

1.3 Study Area

In Nova Scotia, Canada, WNS surveillance has relied on winter survey data. The arrival of *Pd* during the winter of 2010-2011 and declines in the U.S. led the province to request an emergency assessment of hibernating bat species (COSEWIC 2012). By 2013, there was a combined 93% decline of *Myotis lucifugus and M. septentrionalis* at the 5 major hibernacula in the central area of the province (Environment Canada 2018). Hibernating bats including the *Myotis* spp. and *Perimyotis subflavus* species make up the majority of the bat community (Taylor 1997; Moseley 2007) with relatively less migratory species including *Lasiurus cinereus, Lasiurus borealis, Lasionycteris noctivagans* and likely few to none *Eptesicus fuscus* (Taylor 1997; Broders et al. 2003; Moseley 2007; Lucas and Hebda 2011; Rankin 2017).

Pre-WNS to post-WNS monitoring of the summer community of bats in Nova Scotia has been limited to monitoring at two sites. Segers and Broders (2014) found a 99.15% decline of bat activity in Colchester county from 2012 to 2013. In Kejimkujik National Park and National Historic Site (KNPNHS), netting during the summer of 2019 resulted in 71.2% fewer capture compared to historic rates of all species (Grottoli and Broders, unpublished data). However, an acoustic dataset sampling across a 22,000 km^2 region of southwest Nova Scotia during the summers of 2005-2006 can serve as baseline for bat activity before the documentation of WNS and provides the opportunity to assess the decline at a large geographic scale (Farrow and Broders 2011).

Comparatively, the 2005/2006 dataset samples across a substantially larger region than other acoustic studies assessing WNS-related activity changes during the breeding season, e.g., <500 km^2 (Brooks 2011; Dzal et al. 2011; Nocera et al. 2019a). In addition, using this dataset and replicating sampling regimes will provide information on an area of the province where there are few known hibernation sites (Taylor 1997; Randall and Broders 2014). The technical challenge of long-term PAM in this area is the acoustic equipment used in 2005-2006 has now been discontinued and replaced by newer models. This issue will also present itself for other studies as their PAM equipment phases out. More research is needed to address the variation related to the choice of equipment (Adams et al. 2012) and how to compare data sets collected by different PAM setups.

1.4 Objectives

The aim of this study was to assess long-term population trends of bats in southwest Nova Scotia by increasing comparability of sampling collected by different PAM setups. My objectives were 1) to quantitatively characterize variation in the number of species detections between an old and newer model of PAM equipment to compute a correction factor among units; 2) compare the magnitude of activity between successive monitoring periods collected by different equipment using species-specific correction factors and 3) evaluate the management implications of the observed long-term population trends. I expected that there would be more detections by the newer PAM equipment setup and that developing a correction factor would increase our ability to compare data sets. I also predicted that bat activity would be decline for all hibernating species due to the impact of WNS. I addressed these objectives by exploring the difference between two bat acoustic monitoring systems deployed side by side, using the results to calculate species-specific correction factors and applying the corrections to long-term sampling. This will increase our ability to use acoustic monitoring as a method to track long-term population trends.

Chapter 2
Long-term Decline in Bat Activity Using Passive Acoustic Monitoring and an Equipment Correction Factor in Nova Scotia, Canada

2.1 Introduction

Population monitoring is central to conservation biology to detect trends and inform management decisions. Monitoring programs are generally the most effective means of assessing population changes as they are based on repeated monitoring conducted long-term and consistent methodology over large geographic areas. For example, to track population trends of birds, roadside point counts are conducted at the same sites each year during breeding season through the North American Breeding Bird Survey (BBS; Sauer et al., 2017). BBS data has used for federal species assessments, the State of North American Birds Report (Hudson et al. 2017) and as a framework for other monitoring programs such as the North American Amphibian Monitoring Program (NAAMP; Weir et al., 2014). Long-term monitoring programs may be the most reliable source of population data for some species, but this can be time consuming, require many observers and expensive depending on the survey method (Stem et al. 2005; Gunzburger 2007; Paprocki et al. 2014; Villena et al. 2016).

The use of acoustic surveys in conservation programs is increasing in popularity as it is relatively efficient and low cost compared to other techniques (Teixeira et al. 2019). Passive Acoustic Monitoring (PAM) using autonomous recording units provides a means to sample multiple sites and habitats simultaneously and unattended (Heinicke et al. 2015; Campos-Cerqueira and Aide 2016). PAM may perform as well or better than techniques like point counts or netting for inventorying species (O'Farrell and Gannon 1999; Furnas and Callas 2015; Darras et al. 2018) and is useful for studying species that are cryptic (Williams et al. 2018). Acoustic data can provide information on species composition, distribution and behavior, but is most often used to assess changes in activity levels of populations and communities (Sugai et al. 2019). Abundance may be estimated from acoustic data when individuals have vocal signatures (Salmi et al. 2014; Petrusková et al. 2016) or when microphone arrays are used to localize different individuals (Blumstein et al. 2011). Although individuals of many species cannot yet be identified by their calls or without the aid of another method of individual identification (Browning et al. 2017). For long-term monitoring, the change in activity patterns between successive sampling periods can, in some cases, be used as a proxy for population trends (Nocera et al. 2019a).

There are few acoustic monitoring programs that are long-term and cover a large-geographic scale (Jones et al. 2013; Barlow et al. 2015; Loeb et al. 2015) due to the current challenges associated with advancing technology, data management and analyses (Browning et al. 2017). Of these issues, advancing technology has become more challenging to manage for two main reasons. First, there are an growing number of equipment options (Browning et al. 2017) that have varying capabilities in their detection performance. For example, there are differences in the performance of PAM equipment for birds (Rempel et al. 2013; Turgeon et al. 2017) amphibians (MacLaren et al., 2018) and bats (Adams et al. 2012). Second, direct comparison of results from different models might not be possible because of variation resulting in differences in the quality and quantity of acoustic data between data sets both within and across studies. This means there is no solution to account for equipment variation if equipment used in baseline sampling either wears out or is replaced by newer models and becomes unavailable. Furthermore, using equipment from the baseline data set does not take advantage of newer technology with improved or different detection capabilities (Rempel et al. 2013). For example, newer technology may increase the detection distance (Adams et al. 2012) and improve the microphone sensitivity (MacLaren et al. 2018),

Research examining variation in acoustic data due to equipment changes has mainly focused on specific components of the PAM equipment setup. Among recording devices, differences in the number of species detected and number of detections per species have been documented. Recorder performance varies by brand and models of the same brand when presented real vocalizations, playback calls and pure tones (Venier et al. 2012; Adams et al. 2012; Rempel et al. 2013; MacLaren et al. 2018). Other components of a PAM equipment setup also contribute to variation in acoustic data such as the microphone. Research on equipment variation primarily exists for bats as they are the most researched taxa using acoustics (Sugai et al. 2019) given they can be difficult to study through other methods as they are active at night, challenging to catch and emit calls above the human hearing range. Variation in results from microphones can be explained by type (Kaiser and O'Keefe 2015), orientation (Weller and Zabel 2002), weatherproofing (Britzke et al. 2010) and position (Horton et al. 2015; Loeb et al. 2020). For example, the specific microphone type and detection radius can influence the distance at which a specific frequency and species is detected (Ratcliffe and Jakobsen 2018). Overall, the body of research on variability associated with the choice of equipment and how it is used in combination with other components of the PAM setup makes direct comparisons between data sets and studies uncertain.

Given the complexities related to equipment variation, during the data collection phase the current best practice is to have consistent methodology during each successive monitoring period (Frick 2013). Using the same equipment during each sampling period, repeating the methodology and checking the equipment regularly for malfunctions avoids having to account for changes to the setup. Specifically, this could mean redeploying the same brand of detector and microphone, even the same unit of model (Larson and Hayes 2000), between years (Dzal et al. 2011; Sidie-Slettedahl et al. 2015) and sites (Willacy et al. 2015; Measey et al. 2017). PAM equipment must also be checked regularly to ensure it is operating within specifications as it will degrade over time (Turgeon et al. 2017). Following these practices enables researchers to track population trends and compare activity across studies (Ford et al. 2011; Furnas and Callas 2015; Willacy et al. 2015; Nocera et al. 2019a). However, if the PAM setup was to change between data sets (e.g., Nocera et al., 2019), some authors (Rempel et al., 2013 and Loeb et al., 2015), advise making direct comparisons of PAM equipment setups to allow for adjustments accounting for the differences between setups. To my knowledge, there is no research investigating correction factors to account for PAM equipment variation.

The technical challenges of long-term PAM limit our ability to use this method to assess population trends. Practical, efficient and effective sampling is needed to study the impact of threats such as emerging diseases impacting multiple taxa (Skerratt et al. 2007; Forzán et al. 2010; Turner et al. 2011). In North America, acoustic monitoring of bats has been used to assess the impact of the fungus causing White Nose Syndrome (WNS) on summer populations (Brooks, 2011; Dzal et al., 2011; Tomás Nocera et al., 2019). In eastern Canada, declines up to 94% have been observed for hibernating species at overwintering sites attributable to WNS (COSEWIC 2013) but PAM has yet to be used to study long-term summer population trends over a large-geographic scale. Sampling conducted in this region prior to the detection of WNS can serve as baseline activity levels (e.g., Farrow & Broders, 2011). Although, in the case where acoustic equipment used in baseline sampling has been phased out and successive monitoring periods have been conducted using newer technology, comparing data sets may lead to spurious conclusions. Issues related to transitioning to different acoustic equipment will also likely present itself for other studies as their PAM equipment used in baseline wears out or is replaced by newer versions of the same model. More research is needed to address the variation related to the choice of equipment and how to compare data sets collected by different PAM setups.

The aim of this study was to assess long-term population trends of bats in southwest Nova Scotia using data collected using different PAM setups and a calibration factor to correct for variation among setups. My objectives were 1) to quantitatively characterize variation in the number of species detections between an old and newer model of PAM equipment to compute a correction factor among units; 2) compare activity between successive monitoring periods collected by different equipment using species-specific correction factors and 3) evaluate the management implications of the observed long-term population trends. I expected that there would be more detections by the newer PAM equipment setup and that developing a correction factor will increase our ability to compare data sets. I also predicted that bat activity will decline for all hibernating species given the impact of WNS. I addressed these objectives by exploring the difference between two bat acoustic monitoring systems deployed side by side, using the results to calculate species-specific correction factors and applying the corrections to long-term sampling. Exploring acoustic equipment variation will increase our ability to use acoustic monitoring as a method to track long-term population trends.

2.2 Methods

2.2.1 Long-term Acoustic Sampling

2.2.1.1 Baseline Data Set

My study was conducted in southwest Nova Scotia, Canada where the majority of the bat community consists of *Myotis lucifugus, M. septentrionalis,* and *Perimyotis subflavus* (Taylor 1997; Broders et al. 2003; Moseley 2007). Hibernation sites used by these species have been identified in the central portion of the province with few sites known in other areas (Taylor 1997; Moseley 2007; Randall and Broders 2014). There are small numbers of migratory *Lasiurus cinereus, Lasiurus borealis, Lasionycteris noctivagans* and few, if any *Eptesicus fuscus* (Taylor 1997; Lucas and Hebda 2011; Rankin 2017). Baseline acoustic sampling was first conducted across a 22,000 km^2 region in the study area in 2005 and 2006 using the Anabat II paired with the Stainless Steel Microphone

(Titley Electronics) by Farrow & Broders, 2011. Generations of the Anabat have been widely used over several decades for PAM of bats (O'Farrell et al. 1999; Johnson et al. 2002; Skalak et al. 2012; Mtsetfwa et al. 2018; Nocera et al. 2019a) with more recent models phasing out the Anabat II (e.g., SD1, SD2, Express). The Anabat model of detector records in zero-crossing format which saves the loudest signal detected at a point in time, inherently recording only a single bat at once, loud calls and requiring minimal memory needs compared to other recording formats.

Sampling was conducted at 91 sites during the summers of 2005 (40 sites) and 2006 (51 sites) along forested rivers edges between June 5 and August 19 (Appendix 2-B; Appendix 2-C). Each site was sampled for 6-9 nights for two to three, three-night consecutive sampling periods to account for between night variability of bat activity (Hayes 1997). Following the methods of Larson & Hayes, 2000, the Anabat was calibrated to minimize variation in reception between units (Farrow 2007). Using an ultrasonic signal source, the sensitivity of each unit was set until a clear, continuous signal was detected. The signal was set to an intensity of 100 dB at 5 kHz 5 cm from the speaker and then adjusted to 40 kHz when each detector was positioned 9 m away and dialed to optimize the sensitivity. Each Anabat was positioned 3 m high on a wooden platform in a plastic tote with a polyvinyl chloride (PVC) elbow joint angled 45˚ upwards for waterproofing and parallel to the forest edge (Figure 1, Appendix 2-A; Farrow, 2007). The average daily weather between June 5 and August 19 in 2005 and 2006 was 4.06 mm of rain with a high of 24.2 ˚C and low of 13.9 ˚C (Environment and Climate Change Canada 2019)

Figure 1. Acoustic monitoring setup used to sample bat activity at 91 forested rivers across southwest Nova Scotia in 2005/2006. An Anabat II was placed in tub with a PVC elbow joint for weatherproofing (photo by Lesley Farrow).

2.2.1.2 Resampling Data Set

I repeated the sampling regime conducted in the baseline data set during the summers of 2018/2019 following the same schedule but with an updated PAM setup within the area of the each original site (Appendix 2-B). I sampled on the same nights monitored in 2005/2006 during 2018/2019 (Appendix 2-C). To ensure there was consistent monitoring effort between sites, I repeated two of the possible three sampling periods for a total of six nights. The sampling periods selected for replication were the first two monitored in sequence to allow for an extra sampling period if equipment failures occurred that could take place over the third sampling period. I used the Song Meter SM4BAT-FS (Wildlife Acoustics), which compared to the Anabat, is a more recently available full spectrum acoustic recorder on the market (FS; Blejwas et al. 2014; Kaiser and O'Keefe 2015; Rowse et al. 2016; Tuneu-Corral et al. 2020). There are several generations of the Song Meter (e.g., SM2, SM3, SM4) and models used to sample a variety of taxa (Willacy et al. 2015; Charchuk and Bayne 2018). Full spectrum compared to zero-crossing recorders digitize all sounds at one point in time providing finer resolution of signals including recording multiple bats vocalizing simultaneously. Comparatively, full spectrum recorders are generally more sensitive but have higher memory needs and energy demands in contrast to zero-crossing recorders. As well, full spectrum recordings can be converted to zero-crossing but not vice versa.

Two generations of the Wildlife Acoustics microphones were used in this study including the SMM-U2 and SMM-U1 Ultrasonic Microphone (Wildlife Acoustics). I primarily used the U2 because it is cheaper, waterproof and has improved detection performance. The U2 has a higher signal to noise ratio that results in more and longer recordings with lower noise as reported by the manufacturer compared to the U1 microphone which has more directional sensitivity (Wildlife Acoustics 2020). In the resampling data set, sampling was mainly conducted using the U2 microphone with 20 sites sampled for at least one three night sampling period using the U1 microphone (Appendix 2-C). In 2018, all sites were sampled using the U2 except for two sites located in Kejimkujik National Park and National Historic Site (KNPNHS) and then in 2019, sampling was also mainly conducting using the U2, but in some instances the U1 model was used (see Appendix 2-C for which site and night). During late June to August, some sites (n=18) were sampled using the U1 instead of U2 microphones due to a loss of sensitivity as a result of electrostatic damage. The U2 microphones were replaced and the new units equipped with SMM-U2 Grounding Brackets (Wildlife Acoustics) connected to a 12-gauge grounding wire to prevent electrostatic damage (Wildlife Acoustics 2019). The sensitivity of all microphones was checked before each three night deployment using the Ultrasonic Calibrator (Wildlife Acoustics) following the methods recommended by the manufacturer (Wildlife Acoustics 2020). I verified the performance of both the microphone and full recorder system using the Calibrator in CAL mode and ensuring each unit would read the tone at -38 dB for the U1 and -47 dB for the U2 or higher (more negative) indicating the system operating within specifications.

All microphones were positioned 6 m high on aluminum poles designed for snow removal (Figure 2) and typically within 100 m (median distance = 36.4 m) of the original site. Microphones were repositioned away from the original site and higher to avoid environmental clutter (e.g., trees canopy, bushes) to improve call detection, quality, and reduce noise (Weller and Zabel 2002; Britzke et al. 2013; Loeb et al. 2015). The U2 microphones were positioned perpendicular to the river edge but angled horizontal relative to the ground since this model is waterproof. The U1 microphones were also positioned perpendicular to the river edge and angled 45˚ downwards for waterproofing. The Song Meters were each attached to the base pole (4 poles in total) using bungee cords and programed to record the ultrasonic frequencies emitted by bats in the study area of 15-120 kHz. The audio settings were set to record bats at a distance with a 12 dB Gain and minimum 12 dB Trigger Level. To record ultrasonic frequencies of interest, the 16k High Filter was turned off with a minimum trigger frequency of 15 kHz and the sample rate set to 256 kHz which determines the number of samples per second and must be at least double the highest frequency. Signals were saved if they were longer than 1.0 ms, to a maximum recording length of 15 seconds. The Trigger Window was set to record for 2 seconds after the last signal satisfied the trigger. All recordings were saved as WAV audio file format and the firmware version installed in 2018 was 2.1.1 and in 2019 was 2.22a (Wildlife Acoustics). The average daily weather between June 5 and August 19 in 2018 and 2019 was 2.76 mm of rain with a high of 25.2 ˚C and low of 12.6 ˚C (Environment and Climate Change Canada 2019)

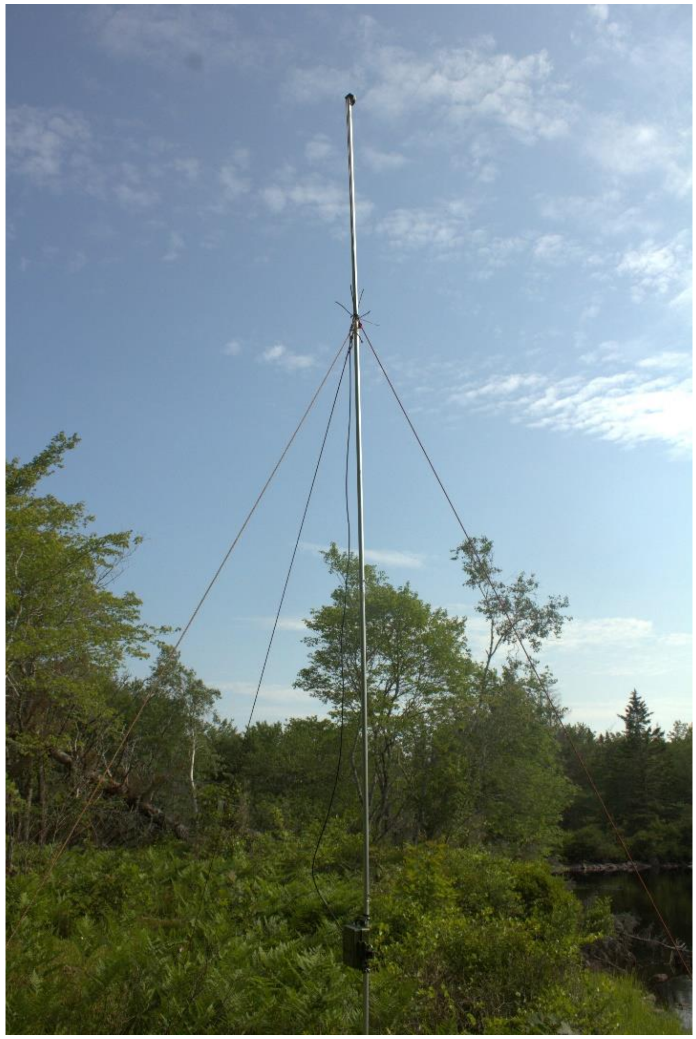

Figure 2. Acoustic monitoring setup used in 2018/2019 to repeat baseline sampling at 91 forested rivers across southwest Nova Scotia conducted in 2005/2006. A Song Meter SM4BAT-FS was setup with the microphone 6 m high on aluminum poles.

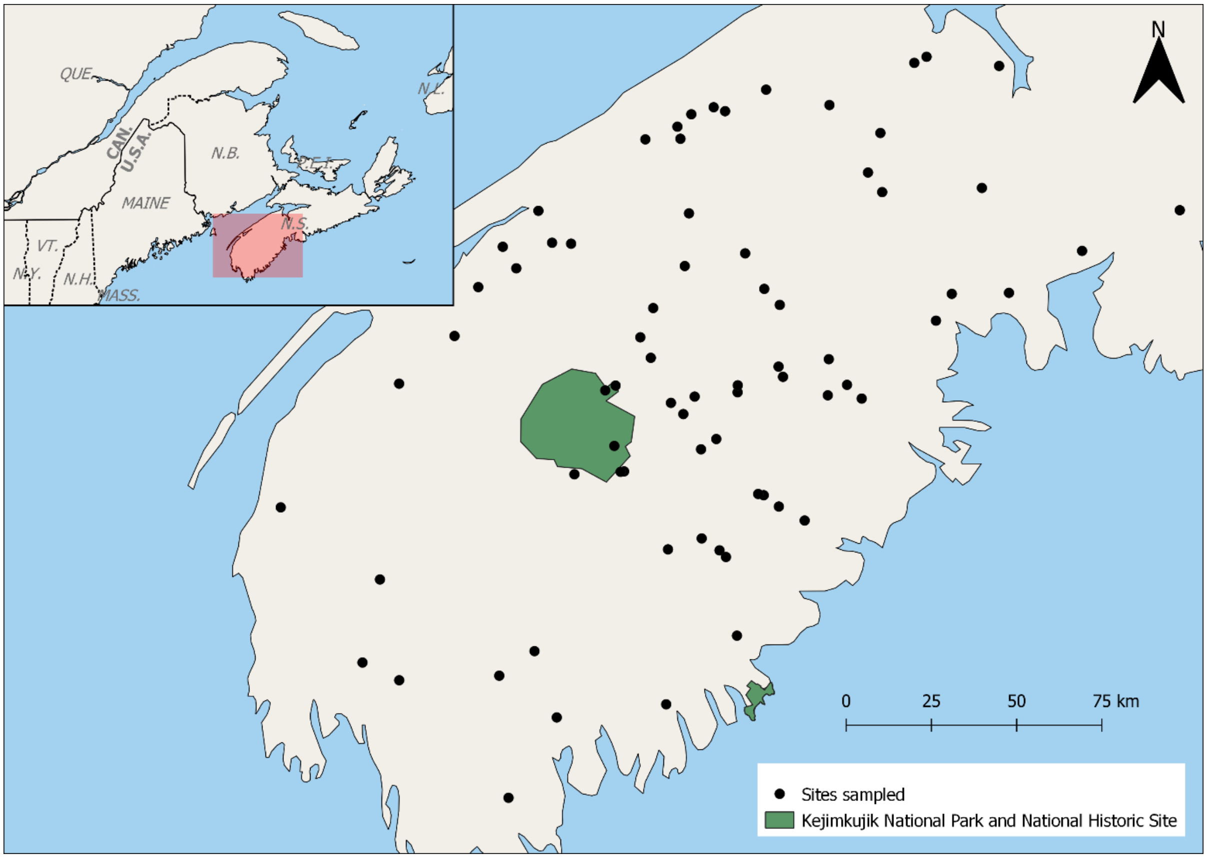

Figure 3. Long-term bat acoustic monitoring sites across southwest Nova Scotia, Canada. Baseline sampling was conducted at 91 sites in 2005/2006 along forested rivers using the Anabat II. Monitoring was repeated at 73 sites in 2018/2019 using the Song Meter SM4BAT-FS.

2.2.3 Species Identification

I reviewed the recordings in each data set for species identification manually using automated identifications tools to assist with sorting recordings. Files were initially processed in Kaleidoscope Pro version 5.1.9 (Wildlife Acoustics) to convert all recordings to zero-crossing format and sort recordings into bat signals that met the following conditions: 15-120 kHz, 1.5-40 ms, max inter-syllable gap of 500 ms and minimum of one pulse with the advanced signal processing feature was turned off. Otherwise, recordings that did not meet the outlined requirements were classified as noise and not further analyzed. The Kaleidoscope automated identification tool was used to assist in species identification using the 5.1.0 Classifier (Wildlife Acoustics). The Auto ID sensitivity was set to Conservative to increase accuracy of identifications. The classifier was set with the region as Nova Scotia and addition of *E. fuscus*. Next, species-specific filters were used as a secondary method to sort recordings by possible species identification in AnalookW version 4.2a (Titley Scientific). I identified recordings manually to species when there were at least three clear search phase calls (single echolocation) that could be differentiated from other species. Calls or sequences (multiple echolocations) that did not meet these requirements were identified as a species grouping or frequency category and excluded from my analyses. I then summarized activity by the number of recordings identified by species.

2.2.4 Comparison of Two Passive Acoustic Monitoring Setups

In 2019, I first compared the PAM equipment setups used in the baseline and resampling data sets to estimate differences in detectability of bat activity. I first quantified the difference in the number of free-flying bat detections when each PAM equipment setup was deployed side-by-side at the same time. I selected the site with the highest bat activity based on the 2018/2019 sampling for the comparison test which was at Eel Weir Bridge (Site #5) along the Mersey River in KNPNHS (Figure

4). Two of the 73 sites were located within the park boundaries including Site #5 and the Mersey River Look-off (Site #4; Figure 4). I compared the Anabat and Song Meter at this site for five nights on August 14-16 and 21-22 recording from 19:00 to 07:00. I used an Anabat SD1 (Titley Scientific) as the most equivalent to the original Anabat II which had been phased out by the manufacturer. The major difference between the Anabat II and SD1 is that storage interface has been amalgamated with the detection unit with no differences in performance reported by the manufacturer. The Anabat was positioned following the methods previously described (Farrow 2007; Farrow and Broders 2011). The unit used in this test was set to a data division ratio of eight indicating the amount of data to be saved with a microphone sensitivity of 6. Alongside the Anabat, I deployed two Song Meters paired with an U1 microphone following the methods previously described (Figure 5). To test the effect of height between the two setups, I positioned one U1 microphone at the same height (3 m) and the second 6 m high and 2 m away to avoid tree canopy (Figure 5). Over the five nights monitored, the mean minimum nightly (19:00-7:00) temperature was 18.48 °C and mean total 24 hr precipitation was 2.26 mm with a notable 11 mm August 21 (Environment and Climate Change Canada 2019).

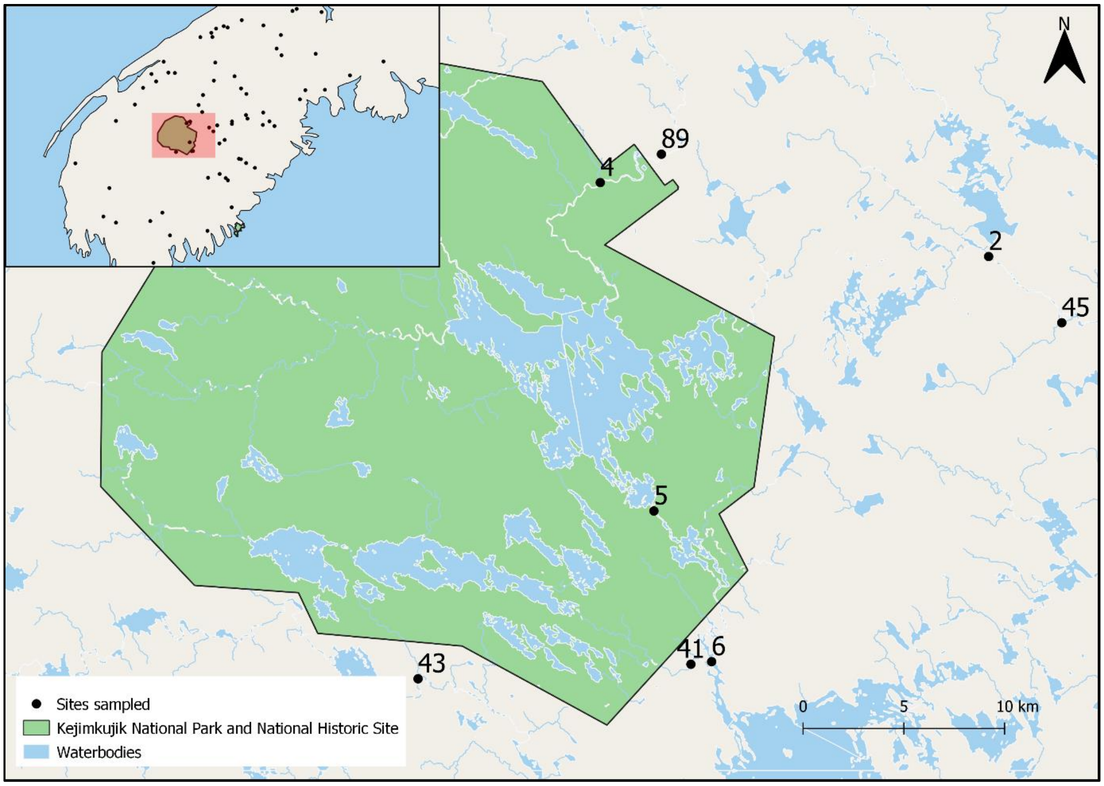

Figure 4. Sites acoustically sampled for bat activity along the Mersey River in southwest Nova Scotia as part of a long-term population monitoring. Two sites were sampled in Kejimkujik National Park and National Historic including Eel Weir Bridge (Site #5) and the Mersey River Look-off (Site #4) where Site #5 was the location of comparison sampling to assess equipment variation.

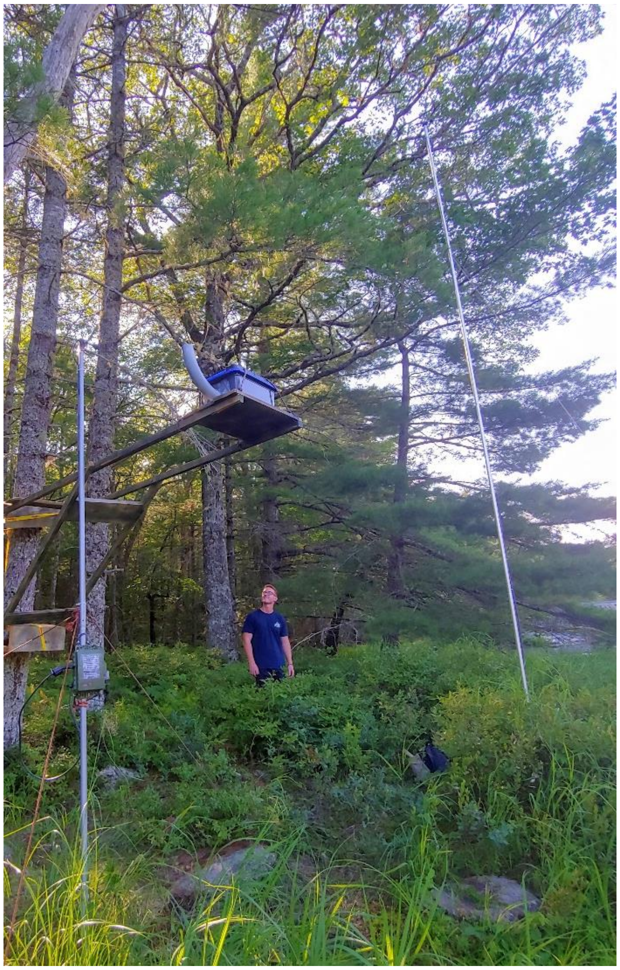

Figure 5. Equipment comparison test of the number of bat detections by two passive acoustic monitoring equipment setups in Kejimkujik National Park and National Historic Site for five nights during August 2020. The Anabat SD1 is positioned on a wooden platform adjacent to a Song Meter SM4BAT with a SMM-U1 microphone at the same height of 3 m and 6 m above ground level on aluminum poles, 2 m away.

Second, I compared the detection distance for each PAM equipment setup using a synthetic tone. In an open field 10 km from KNPNHS, an Anabat and Song Meter were positioned alongside each other mid-day in August 2019. Both setups were positioned 1.5 m above ground level with the microphones angled as previously described (i.e., SD1 in tub with PVC tub angled upwards, U1 angled downwards) and positioned facing the direction of the signal (Appendix 2-B). The unit of Anabat used in this test was tuned to sensitivity of five and a data division ratio of 8. I played a 40 kHz (+/- 10 hZ) tone 1.5 m high at 0˚ using the Ultrasonic Calibrator angled horizontal. The calibrator was set to Chirp Mode which emits a 100 ms signal with an amplitude of 104 dB SPL (+/- 3 dB) every 500 ms. The signal was played for 5 seconds at 5 m intervals (5-30 m). I did not test the effect of multiple frequencies or angles, given the effect of angle was found to be the same for various brands of detectors including two directional and three omnidirectional microphones (Adams et al. 2012) and the majority of the local bat community consists of non-migratory species that echolocate predominantly at 40 kHz. Recordings were converted and reviewed in zero-crossing format in Analook to identify if a clear signal was detected at each distance interval. The Song Meter’s used for both comparison tests had been deployed previously for the two field seasons in 2018/2019. The two Anabat SD1 units used for these comparison tests were manufactured in the early 2000’s and serviced by Titley Scientific prior to data collection to ensure they were operating within specifications.

2.2.5 Equipment Correction Factor

I used the proportion of detections between the Anabat and Song Meter during the five-night comparison test to develop a correction factor to account for equipment variation per species detected and height (3 m or 6 m). Under the assumption the Song Meter is the best detection method, I quantified the number of Anabat detections as though they were successes in making the same detection as the Song Meter. I used R version 3.6.3 (R Core Team 2020) to calculate the mean proportion of successes across nights following a binomial distribution to construct exact confidence intervals (R package ‘binom’ and the function ‘binom.cloglog’; Dorai-Raj 2014). I constructed 95%

confidence intervals using the complementary log parameterization on the observed number of successes using the sum of successes and the sum of independent trials (Dorai-Raj 2014). The probability of success as given by binomial distribution was used as the correction factor. To evaluate the efficacy of each correction factor, I corrected the Song Meter detections collected during the comparison test then examined the difference between the Anabat and corrected Song Meter detections using the Root Mean Square Error (RMSE) and mean errors.

To estimate activity changes between the baseline and resampling data sets, I applied each species-specific correction factor between the 3 m Anabat and the 6 m Song Meter comparison given these mimic the setups used in the long-term SWNS data sets. To compare the Anabat to the Song Meter detections, I multiplied the correction factor by the number of detections in the resampling data set which was collected by the Song Meter. I then divided by the number of baseline detections collected by the Anabat multiplied by 100 to calculate the percent change and subtracted from 100 to assess if the change was a decline.

$$Change\ in\ activity = \left(\frac{Resampling\ detections * Correction\ factor}{Baseline\ detections} * 100\right) - 100$$

I applied each species correction factor to the site where they were developed in KNPNHS with a U1 microphone (Site #5; Eel Weir Bridge). I then applied the correction factor to sampling at all other sites. I applied the correction factor to sampling upstream in KNPNHS (Site #4; Mersey River Look-off) relative to Site #5 and then downstream (Site #6; Rossignol Bridge; Figure 4) located just outside the park boundary. Site #4 was also equipped with a U1 microphone in the resampling data set but 100 m from the baseline site. Site #6 was set up with a U2 microphone in the resampling data set and 162 m from the site sampled in the baseline data. Finally, I applied the correction factor to the whole resampling data set across southwest Nova Scotia including sampling at all 73 sites.

Most sites sampled with the U2 microphone and with a median distance of 36.4 m from the original sites.

2.3 Results

I analyzed a total of 876 nights (73 sites x 12 nights) of acoustic data from sampling collected across southwest Nova Scotia during 2005/2006 and 2018/2019. I identified the presence of six species including *M. lucifugus, M. septentrionalis, P. subflavus, L. cinereus, L. noctivagans* and *L. borealis* (Appendix 2-D). Of these species, *M. lucifugus* and *P. subflavus* were detected over five nights of equipment comparison sampling at KNPNHS during August 2019. Comparing the number of detections per species over five nights (Table 1), the Anabat detected relatively less activity compared to the Song Meter. The Song Meter paired with the U1 microphone detected more bat activity at the same height (*M. lucifugus*=539, *P. subflavus*=728) and when positioned 3 m higher (*M. lucifugus*=1,038, *P. subflavus*=3,290) compared to the Anabat (*M. lucifugus*=22, *P. subflavus*=86; Table 1). When estimating the detection distance of each system, I found a 40 kHz tone was detected up to 10 m away for the Anabat, and up to 15 m for the Song Meter paired with the U1 microphone.

Table 1. Comparison of the number of bat detections of three side-by-side bat acoustic monitoring setups at Kejimkujik National Park and National Historic Site for five nights of sampling during August 2019. An Anabat at 3 m was compared to the Song Meter with a U1 microphone at 3 m (A) and at 6 m (B) above ground level.

	Myotis lucifugus			***Perimyotis subflavus***		
Night	**Anabat 3 m**	**Song Meter 3 m**	**Song Meter 6 m**	**Anabat 3 m**	**Song Meter 3 m**	**Song Meter 6 m**
1	0	152	224	28	225	1,399
2	1	37	82	8	363	1,096
3	4	114	342	7	44	443
4	11	174	290	11	52	179
5	6	62	100	32	44	173
Total	22	539	1,038	86	728	3,290

Correction factors with 95% confidence intervals were developed for *M. lucifugus* and *P. subflavus* at 3 m and 6 m (Table 2). For *M. lucifugus*, the correction factor at 3 m was 0.050 (0.034, 0.070) and 0.026 (0.017, 0.037) at 6 m. For *P. subflavus*, the correction factor was 0.124 (0.102, 0.149) at 3 m and 0.028 (0.022, 0.034) at 6 m. The correction factors were larger, indicating a greater probability of success, when the Anabat and Song Meter were positioned at the same height than when the Song Meter was positioned higher. The probability of success was higher for *P. subflavus* at 3 m than *M. lucifugus* but similar for both species at 6 m.

Table 2. Correction factors (CF) with 95% confidence intervals to account for equipment variation in acoustic sampling conducted in southwest Nova Scotia. Correction factors were developed to compare A) the relative performance of the Anabat at 3 m to the Song Meter at 3 m and B) the relative performance of the Anabat at 3 m to the Song Meter at 6 m.

	Correction Factor*	**Lower Confidence Limit**	**Upper Confidence Limit**
Myotis lucifugus			
3 m	0.050	0.034	0.070
6m	0.026	0.017	0.037
Perimyotis subflavus			
3 m	0.124	0.102	0.149
6 m	0.028	0.022	0.034

*Correction factors were developed using the probability of success as given by binomial distribution with 95% confidence intervals. The Song Meter was assumed to be the best detection method and the number of Anabat detections were quantified as though they were successes in making the same detection as the Song Meter

I compared the predicted number of Song Meter detections to the Anabat detections to assess the efficacy of the correction factors (Table 3). For example, the Song Meter detections at 6 m of *M. lucifugus* were 224, 82, 342, 290 and 100 (Table 1) became 5.825, 2.149, 8.879, 7.533, and 2.615 when the correction factor was applied (Table 3). The RMSE of the predicted Song Meter values were approximately ±4 detections of *M. lucifugus* at 3 m and 6 m (Table 3). For *P. subflavus*, the RMSE of the predicted Song Meter values was approximately ±20 at 3 m and ±17 at 6 m. The mean of the raw errors for both heights and species were centered on zero indicating no systematic bias (Table 3).

Table 3. Comparison of the predicted Song Meter detections to the Anabat detections per species and height to assess the efficacy of the correction factors. The Root Mean Square Error (RMSE) and mean of the errors with 95% confidence intervals were used quantify the difference between the Anabat and prediction Song Meter detections. The Anabat at 3 m was compared to the Song Meter at 3 m (A) and at 6 m (B).

Species	A	B
Myotis lucifugus		
1	7.594 (5.135, 10.73)	5.825 (3.936, 8.298)
2	1.886 (1.275, 2.666)	2.149 (1.452, 3.061)
3	5.708 (3.860, 8.068)	8.879 (6.000, 12.65)
4	8.686 (5.874, 12.28)	7.533 (5.091, 10.732)
5	3.127 (2.115, 4.420}	2.615 (1.767, 3.725)
RMSE	3.741 (4.008, 4.719)	3.938 (4.124, 5.005)
Mean Error	$-2.220\text{x}10^{-16}$ (-1.748, 2.233)	$-8.864\text{X}10^{-17}$ (-1.751, 2.293)
Perimyotis subflavus		
1	28.06 (22.94, 33.72)	38.67 (31.39, 47.08)
2	45.19 (36.95, 54.30)	30.30 (24.60, 36.89)
3	5.587 (4.567, 6.713)	12.26 (9.96, 14.93)
4	6.580 (5.379, 7.907)	4.971 (4.036, 6.054)
5	5.587 (4.567, 6.713)	4.805 (3.901, 5.852)
RMSE	20.48 (18.34, 23.60)	16.79 (15.25, 19.62)
Mean Error	$7.098\text{x}10^{-16}$ (-3.320, 3.670)	$-1.783\text{x}10^{-16}$ (-3.424, 3.963)

The detections of *M. lucifugus* and *P. subflavus* sampled during 2018/2019 by the Song Meter were corrected for equipment variation (Table 4). For example, the Song Meter detections of *M. lucifugus* collected during the resampling period at Site 5, 4, 6 and collectively across SWNS were 2138, 32, 93, and 10876 then once the correction factor was applied became 55.37, 0.829, 2.409, and 281.68, respectively (Table 4).

Table 4. Estimated change in bat activity at a subset of sites along the Mersey River and across 73 sites in southwest Nova Scotia (SWNS). Baseline acoustic sampling was conducted in 2005/2006 with the Anabat and repeated in 2018/2019 with the Song Meter. Detections in the raw resampling data set were corrected for equipment variation using correction factors (CF) developed at Kejimkujik National Park and National Historic Site: *Myotis lucifugus* CF=0.026 95% CI (0.017, 0.037) and *Perimyotis subflavus* CF=0.028 95% CI (0.022, 0.034).

Site	Baseline Sampling (2005/2006)	Resampling (2018/2019)		
		Raw	Corrected	95% CI
Myotis lucifugus				
Site #5	183	2,138	55.35	(37.40, 78.85)
Site #4	70	32	0.829	(0.560, 1.181)
Site #6	27	93	2.408	(1.627, 3.432)
Across SWNS	6,255	10,876	281.5	(190.3, 401.1)
Perimyotis subflavus				
Site #5	34	556	15.36	(12.47, 18.70)
Site #4	43	6	0.166	(0.135, 0.202)
Site #6	10	35	0.967	(0.785, 1.177)
Across SWNS	349	1,091	30.13	(24.46, 36.69)

Activity of *M. lucifugus* and *P. subflavus* declined dramatically at three sites and across all sites between 2005/2006 to 2018/2019 (Table 5). Across SWNS, *M. lucifugus* declined by 95.5% 95% CI (96.96%, 93.58%) and *P. subflavus* by 91.37% 95% CI (93.00%, 89.50%). Activity at Site #5 which had the most bat activity of hibernating species during 2018/2019 sampling and where the correction factors were developed, declined comparatively less between sampling periods than Site #4 upstream in KNPNHS and outside of the park downstream at Site #6.

Table 5. Change in bat activity with 95% confidence intervals (CI) across southwest Nova Scotia (SWNS) from 2005/2006 to 2018/2019 at a subset of sites along the Mersey River and across 73 sites. Site #5 is where equipment correction factors were developed while #4 is upstream and #6 is downstream relative to this site.

Site	Change in Activity (%)	Lower CI	Upper CI
Myotis lucifugus			
Site #5	-69.76	-79.56	-56.91
Site #4	-98.82	-99.20	-98.31
Site #6	-91.08	-93.97	-87.30
Across SWNS	-95.50	-96.96	-93.59
Perimyotis subflavus			
Site #5	-54.84	-63.33	-45.00
Site #4	-99.61	-99.69,	-99.53
Site #6	-90.33	-92.15	-88.23
Across SWNS	-91.37	-92.99	-89.49

2.4 Discussion

The difference in activity detected by the Anabat compared to the Song Meter demonstrates, unsurprisingly, that direct comparisons of activity sampled by different PAM equipment setups cannot be made within a study or between studies. The two setups examined in this study differed in activity detected by species, night, and height providing evidence for the usefulness of an overall equipment correction factor to account for the sum of this variation. There was reasonable confidence in the correction factors as there was no systematic bias in the values and activity changes of *M. lucifugus* and *P. subflavus* reflected dramatic declines attributable to WNS observed from other survey techniques in the region and across North America (COSEWIC 2013). Furthermore, the differences in performance between the two PAM equipment setups align with other studies (Adams et al., 2012).

The population trends detected align with regional declines observed through changes in winter and summer colony counts and capture rates. Across eastern Canada, *M. lucifugus* and *P. subflavus* have declined by up to 94% and 75% respectively at overwintering sites while a handful of pre-WNS to post-WNS *M. lucifugus* maternity colony counts in Ontario and Quebec have declined by up to 99% (COSEWIC 2013). In Nova Scotia, winter colony counts of *Myotis spp.* at five major hibernacula declined by 93% two years after the initial detection of WNS in 2011 (Nova Scotia Department of Lands and Forestry 2011; COSEWIC 2013). However, there are no specific estimates of *P. subflavus* in Nova Scotia given the low number of historic and recent observations at hibernation sites (COSEWIC 2013). As for summer monitoring, Segers & Broders, 2014, reported a 99.15% decline of *Myotis* acoustic activity between 2012-2013 in the province. Netting from the summer of 2019 at KNPNHS revealed a decline of captures per unit effort of 56% for *P. subflavus* and 67.9% for *M. lucifugus* compared to historic rates (Grottoli and Broders, unpublished data; Poissant et al., 2010).

Comparing population trends between monitoring techniques, changes in acoustic activity reflect the magnitude of the decline observed through changes in colony counts and capture rates. In general, population trends of *M. lucifugus* at KNPNHS were similar between acoustic activity (-69.76% 95% CI (-79.56, -56.91)) and captures (-67.9%) with acoustic activity across SWNS (-95.50 95% CI (-96.96, -93.59)) reflecting changes in overwintering colony counts (-94%). For *P. subflavus,* population trends were comparable between acoustic activity patterns at KNPNHS (-54.84% 95% CI (-63.33, -45.00)) and changes in captures (-56%) but acoustic activity across SWNS (-91.37 95% CI (-92.99, -89.49)) was more dramatic than observations at overwintering colony counts in eastern Canada (-75%). Population decline of both species was lower at Eel Weir Bridge (Site #5), KNPNHS, than across SWNS although there were only six nights sampled per site per data set. Acoustic sampling is also more appropriate for detecting large than small magnitudes of change. Nonetheless, activity changes at Site #5 may also represent differential survival for these species in this area compared to the landscape. Acoustic activity prior to WNS was relatively high at KNPNHS compared to sampling across the landscape so protected areas such as KNPNHS may represent a significant area for population recovery from WNS. Although both species declined dramatically since the invasion of WNS, there was relatively higher activity of *P. subflavus* compared to *M. lucifugus* in KNPNHS (Site #5) during the equipment comparison test which may be explained by differential activity patterns. Specifically, *P. subflavus* primarily forages over water in this region and throughout the night whereas *M. lucifugus* is more of a dietary generalist and forages over water initially after sunset (Broders et al. 2003). Therefore, activity of *P.* subflavus may be more detectable than *M. lucifugus* activity due to the placement of the equipment near water resulting in a sampling bias.

My study adds to the body of literature showing the long-term impacts of WNS across North America and provides an opportunity to continue to track population changes in eastern Canada. The spatial scale examined in this study is unmatched and shows similar declines post-WNS over comparable time periods in the eastern United States (Brooks 2011; Dzal et al. 2011; Nocera et al. 2019a). My research shows the effect of WNS is persisting eight years since the initial detection of the fungus in this region. Once the most common bat species in Nova Scotia, *M. lucifugus,* has now been greatly reduced across the landscape, including the southwest region of the province away from the five major known hibernacula. This provides evidence that WNS affects the entire bat population in the province, not just individual hibernacula, suggesting the disease is present in counties with no known hibernacula or is affecting bats that spend the summer in this area (Segers and Broders 2015). My research also provides the first summer population assessment of *P. subflavus* in the region since the invasion of WNS. Using this long-term acoustic monitoring data can provide a non-invasive means to track the long-term impacts of the disease.

The relative performance of the Anabat to the Song Meter in my study aligns with the work of Adams et al., 2012, however, our specific recommendations differ. Adams et al., 2012, suggested that depending on the study question and local bat fauna, the Anabat SD2 could perform similar to four other full spectrum detectors including the Song Meter SM2BAT (Wildlife Acoustics). Although in ideal conditions the Anabat may have performance comparable to other brands, in most cases the Anabat had the fewest detections in their tests (Adams et al. 2012). I found that the performance of the Anabat SD1, which I used to model the Anabat II, performed poorly when compared to a full spectrum SM4BAT detector as the Song Meter had a further detection distance and a greater number of detections for two species. The SD2 is not reported by the manufacturer to have improved detection performance compared to the SD1 (Hourigan and Corben 2017) so the II, SD1, and SD2 performance should be comparable apart from other sources of variation (e.g. by unit; Larson and Hayes 2000).

Adams et al., 2012, found that the SD2 may not accurately document activity for higher frequency species given no signals were detected at 85 kHz or 115 kHz. For this study, this may mean *M. septentrionalis* historically found in the province would be under-represented in the baseline data set collected by the Anabat II as this species has relatively high frequency echolocation characteristics (Faure et al. 1993). As for this species in the resampling data set, there were few *M. septentrionalis* detections across southwest Nova Scotia (Appendix 2-D) and no detections during the five nights of comparison sampling in KNPNHS. In the U.S., this species is disproportionately at risk of extinction due to WNS compared to other species (Frick et al. 2015) and may be experiencing similar species-dependent impacts of WNS in this region. Regardless of the impact of WNS or sampling equipment, this species is likely to be underrepresented in acoustic data due to the low intensity calls and placement of the detectors as this species primarily forages within the forest interior (Faure et al. 1993; Broders et al. 2003).

In general, correction factors are useful in wildlife research to account for bias in survey techniques when estimating population size. For example, correction factors accounting for persistence rates of scat (Brodie 2006), visibility of animals during aerial surveys (Pearse et al. 2008), and detection distance per species (Monadjem et al. 2017) have increased the accuracy of estimates. In this study, decline of bat activity could not be explained solely by other factors such as the natural high variability of bat activity across nights (Hayes 1997; Broders 2003). There are many sources of variation to account for in acoustic sampling not limited to the movement of sound through air, call characteristics and equipment (Adams et al. 2012). Within equipment variation, there are a number of factors contributing to an overall equipment variation such as the microphone type, height and distance to clutter. Monadjem et al., 2017, explored differences in detection distance of bats by one recording system and developed correction factors to compare activity levels between species. However, to develop correction factors per species per detection systems using their method requires recording hand released individuals at varying distances which is not practical for resource managers.

Other approaches to account for equipment variation that do not require handling are to examine specific factors like the microphone signal to noise ratio, directionality, and sensitivity as suggested by Rempel et al., 2013. By simply placing each setup side by side, these factors may not be specifically identified but can be accounted for. There was no apparent sampling bias in the correction factors, although a larger correction was required at 6 m than 3 m. Positioning the Song Meter higher increased the number of detections and thus suggests that maintaining the same height between data sets will improve comparability. Another consideration is how long to conduct side by side sampling for, where Loeb et al., 2015 suggest comparing equipment for one season. This would likely decrease the width of my confidence intervals and increase the precision of my equipment correction factors. Although, comparing population trends between monitoring techniques provides another means to assess the validity of correction factors and activity estimates. Sampling for a whole season may be impractical given the large amount of data typical of acoustic sampling and the capabilities of older equipment. In the case of the Anabat SD1 model used in this study, batteries need to be replaced every three days. In areas where classifiers are appropriate to automatically identify recordings and recorders can be deployed for extended periods of time, sampling for a whole field season may be practical.

2.5 Management Implications

The results of this study demonstrate that equipment variation needs to be accounted for to assess long-term changes in activity patterns of species as technology advances. Wildlife managers need to plan for how they will account for equipment variation when adjustments to the setup and how the equipment is used are made. For example, developing a systematic protocol for long-term sampling in the case of taxa where large-scale acoustic monitoring programs have yet to be developed or in the case of bats, following standardized protocols designed by the North American Bat Monitoring program (Loeb et al. 2015). A simple technique to estimate the differences between PAM setups is to deploy them side by side to calculate species-specific correction factors. Correction factors can be evaluated and activity trends compared to other monitoring techniques to assess their efficacy. Precision of the correction factors may increase by sampling for more than five nights and potentially a whole field season as suggested by Loeb et al., 2015. Further research is needed to evaluate the performance of different equipment and the efficacy of equipment correction factors across studies.

As for monitoring bat populations and the impact of WNS, I showed that long-term acoustic monitoring is a viable technique in consideration of technological challenges associated with PAM and the desire for non-invasive monitoring of endangered species. Long-term monitoring needs to continue to further our understanding of the summer population trends of bats attributable to WNS and the larger group of threats they face including habitat degradation, fragmentation and wind energy development (COSEWIC 2013). Managers can use acoustic data in conjunction with other monitoring techniques to assess population trends in areas with no known hibernation sites, for species difficult to study in hibernacula (e.g., *P. subflavus*) and to identify areas that may be significant for WNS recovery such as protected areas. This long-term SWNS data set may be used to continue to track summer population changes of *M. lucifugus* and *P. subflavus*, however monitoring better suited to detect *M. septentrionalis* is needed. KNPNHS may serve as an important area for these species during WNS recovery and periodic monitoring should continue to document population changes.

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Appendix A

Anabat Acoustic Monitoring System

Appendix 2-A. Close up of Anabat II acoustic monitoring system used in sampling during 2005-2006 in southwest Nova Scotia, Canada. Detector with microphone placed in tub with a PVC elbow joint for weatherproofing (photo by Lesley Farrow).

Appendix B

Long-term Southwest Nova Scotia Acoustic Sampling Sites

Number of Sites	Site ID	Grid Zone	2005/2006		2018/2019	
			Easting	Northing	Easting	Northing
1	1	20T	350455	4921742	350406	4921731
2	2	20T	336422	4919814	336378	4919861
3	3	20T	332199	4929350	332398	4929362
4	4	20T	322623	4922742	322666	4922832
5	5	20T	324256	4911181	324257	4911183
6	6	20T	326306	4905719	326166	4905800
7	7	20T	369663	4928191	369671	4928221
8	8	20T	376426	4919976	376410	4919877
9	10	20T	392213	4936006	392261	4935836
10	11	20T	342537	4910053	342452	4910007
11	12	20T	355536	4900040	355413	4900100
12	13	20T	345724	4888972	345839	4888754
13	14	20T	349042	4870769	349106	4870762
14	15	20T	333799	4856694	333787	4856672
15	16	20T	407508	4938959	407607	4941387
16	17	20T	299714	4837799	299802	4837864
17	18	20T	310586	4854411	310534	4854527
18	19	20T	298722	4863633	298617	4863675
19	20	20T	306159	4868665	306227	4868626
20	21	20T	274110	4884713	274064	4884721
21	22	20T	277358	4863431	277414	4863395
22	23	20T	269655	4867408	269770	4867390
23	24	19T	252411	4900704	732998	4900096
24	25	20T	279533	4925510	279489	4925552
25	26	20T	291445	4935032	291425	4935105
26	27	20T	296711	4945146	296719	4945121
27	28	20T	302129	4953279	302097	4953337
28	29	20T	309848	4960389	309751	4960581
29	30	20T	332468	4974769	332433	4974762
30	31	20T	339746	4974650	339732	4974696
31	32	20T	339977	4948232	339983	4948285
32	33	20T	352710	4950558	352658	4950620
33	34	20T	370908	4980965	370864	4980975

34	36	20T	381164	4975122	381347	4974986
35	37	20T	391167	4990523	391213	4990564
36	39	20T	423645	4948845	422995	4949907
37	40	20T	443347	4958144	0443463	4958144
38	41	20T	325550	4905929	325427	4905728
39	43	20T	315714	4905475	315707	4905479
40	45	20T	338902	4917470	338916	4917459
41	47	20T	334991	4889217	334989	4889227
42	48	20T	342133	4891359	342144	4891349
43	49	20T	347186	4887361	347166	4887333
44	51	20T	341411	4921025	341383	4921050
45	52	20T	350477	4923183	350477	4923183
46	53	20T	345691	4912084	345710	4912078
47	54	20T	354301	4900375	354249	4900387
48	55	20T	358563	4897666	358545	4897658
49	56	20T	363905	4894655	363915	4894620
50	57	20T	359993	4924761	359997	4924744
51	58	20T	359645	4939778	359641	4939743
52	59	20T	356495	4943127	356478	4943143
53	62	20T	395662	4941352	395658	4941357
54	63	20T	359113	4926908	359113	4926894
55	64	20T	369312	4920700	369308	4920695
56	65	20T	373389	4922796	373386	4922799
57	67	20T	402249	4963239	402281	4963250
58	73	20T	378624	4966842	378615	4966850
59	74	20T	388611	4989334	388648	4989357
60	76	20T	406251	4988435	406239	4988435
61	77	20T	381484	4962747	381491	4962736
62	78	20T	357788	4984418	357781	4984432
63	79	20T	349164	4980176	349158	4980176
64	80	20T	346823	4981036	346802	4981052
65	81	20T	342145	4979696	342122	4979710
66	82	20T	339177	4977226	339166	4977212
67	84	20T	341133	4959182	341133	4959200
68	86	20T	312406	4953871	312410	4953872
69	87	20T	316361	4953544	316371	4953554
70	88	20T	304798	4948774	304781	4948779
71	89	20T	324862	4923765	324862	4923774
72	90	20T	330326	4933716	330308	4933693
73	91	20T	333157	4939717	333160	4939714

Appendix 2-B. UTM coordinates of forested river sites (n = 73) acoustically monitored for bat activity in 2005/2006 and 2018/2019 across southwest Nova Scotia. Resampling was conducted typically <100 m away from the original site with a median distance of 36.4 m from each original site. Sites not successfully sampled for six nights on the same nights in both data sets (n = 17) were removed from analyses.

Appendix C

Long-term Southwest Nova Scotia Bat Acoustic Sampling Schedule

Number of Sites	Site ID	Year Sampled	Night 1	Night 2	Night 3	Night 4	Night 5	Night 6
1	1	2005/2018	12-Jul	13-Jul	14-Jul	09-Aug	10-Aug	11-Aug
2	2	2005/2018	12-Jul	13-Jul	14-Jul	09-Aug	10-Aug	11-Aug
3	3	2005/2018	12-Jul	13-Jul	14-Jul	09-Aug	10-Aug	11-Aug
4	4	2005/2018	12-Jul	13-Jul	14-Jul	09-Aug	10-Aug	11-Aug
5	5	2005/2018	12-Jul	13-Jul	14-Jul	09-Aug	10-Aug	11-Aug
6	6	2005/2018	22-Jul	23-Jul	24-Jul	09-Aug	10-Aug	11-Aug
7	7	2005/2018	24-Jun	25-Jun	26-Jun	18-Jul	19-Jul	20-Jul
8	8	2005/2018	24-Jun	25-Jun	26-Jun	18-Jul	19-Jul	20-Jul
9	10	2005/2018	24-Jun	25-Jun	26-Jun	18-Jul	19-Jul	20-Jul
10	11	2005/2018	22-Jul	23-Jul	24-Jul	12-Aug	13-Aug	14-Aug
11	12	2005/2018	18-Jul	19-Jul	20-Jul	12-Aug	13-Aug	14-Aug
12	13	2005/2018	22-Jul	23-Jul	24-Jul	12-Aug	13-Aug	14-Aug
13	14	2005/2018	27-Jun	28-Jun	29-Jun	22-Jul	23-Jul	24-Jul
14	15	2005/2018	27-Jun	28-Jun	29-Jun	22-Jul	23-Jul	24-Jul
15	16	2005/2018	18-Jul	19-Jul	20-Jul	06-Aug	07-Aug	08-Aug
16	17	2005/2018	30-Jun	01-Jul	02-Jul	25-Jul	26-Jul	27-Jul
17	18	2005/2018	30-Jun	01-Jul	02-Jul	25-Jul	26-Jul	27-Jul
18	19	2005/2018	30-Jun	01-Jul	02-Jul	25-Jul	26-Jul	27-Jul
19	20	2005/2018	30-Jun	01-Jul	02-Jul	25-Jul	26-Jul	27-Jul
20	21	2005/2018	30-Jun	01-Jul	02-Jul	25-Jul	26-Jul	27-Jul
21	22	2005/2018	03-Jul	04-Jul	05-Jul	28-Jul	29-Jul	30-Jul
22	23	2005/2018	03-Jul	04-Jul	05-Jul	28-Jul	29-Jul	30-Jul
23	24	2005/2018	03-Jul	04-Jul	05-Jul	28-Jul	29-Jul	30-Jul
24	25	2005/2018	03-Jul	04-Jul	05-Jul	28-Jul	29-Jul	30-Jul
25	26	2005/2018	03-Jul	04-Jul	05-Jul	28-Jul	29-Jul	30-Jul
26	27	2005/2018	06-Jul	07-Jul	08-Jul	28-Jul	29-Jul	30-Jul
27	28	2005/2018	06-Jul	07-Jul	08-Jul	31-Jul	01-Aug	02-Aug
28	29	2005/2018	06-Jul	07-Jul	08-Jul	31-Jul	01-Aug	02-Aug
29	30	2005/2018	06-Jul	07-Jul	08-Jul	31-Jul	01-Aug	02-Aug
30	31	2005/2018	09-Jul	10-Jul	11-Jul	31-Jul	01-Aug	02-Aug
31	32	2005/2018	09-Jul	10-Jul	11-Jul	31-Jul	01-Aug	02-Aug

32	33	2005/2018	09-Jul	10-Jul	11-Jul	31-Jul	01-Aug	02-Aug
33	34	2005/2018	15-Jul	16-Jul	17-Jul	03-Aug	04-Aug	05-Aug
34	36	2005/2018	15-Jul	16-Jul	17-Jul	03-Aug	04-Aug	05-Aug
35	37	2005/2018	15-Jul	16-Jul	17-Jul	15-Aug	16-Aug	17-Aug
36	39	2005/2018	22-Jul	23-Jul	24-Jul	06-Aug	07-Aug	08-Aug
37	40	2005/2018	25-Jul	26-Jul	27-Jul	03-Aug	04-Aug	05-Aug
38	41	2005/2018	23-Jun	24-Jun	25-Jun	10-Jul	11-Jul	12-Jul
39	43	2005/2018	10-Jul	11-Jul	12-Jul	31-Jul	01-Aug	02-Aug
40	45	2005/2018	05-Jun	06-Jun	07-Jun	13-Jul	14-Jul	15-Jul
41	47	2006/2019	08-Jun	09-Jun	10-Jun	10-Jul	11-Jul	12-Jul
42	48	2006/2019	23-Jun	24-Jun	25-Jun	10-Jul	11-Jul	12-Jul
43	49	2006/2019	10-Jul	11-Jul	12-Jul	07-Aug	08-Aug	09-Aug
44	51	2006/2019	11-Jun	12-Jun	13-Jun	13-Jul	14-Jul	15-Jul
45	52	2006/2019	16-Jul	17-Jul	18-Jul	03-Aug	04-Aug	05-Aug
46	53	2006/2019	13-Jul	14-Jul	15-Jul	03-Aug	04-Aug	05-Aug
47	54	2006/2019	13-Jul	14-Jul	15-Jul	07-Aug	08-Aug	09-Aug
48	55	2006/2019	11-Jun	12-Jun	13-Jun	13-Jul	14-Jul	15-Jul
49	56	2006/2019	14-Jun	15-Jun	16-Jun	07-Aug	08-Aug	09-Aug
50	57	2006/2019	14-Jun	15-Jun	16-Jun	16-Jul	17-Jul	18-Jul
51	58	2006/2019	17-Jun	18-Jun	19-Jun	19-Jul	20-Jul	21-Jul
52	59	2006/2019	14-Jun	15-Jun	16-Jun	16-Jul	17-Jul	18-Jul
53	62	2006/2019	14-Jun	15-Jul	16-Jul	13-Jul	14-Jul	15-Jul
54	63	2006/2019	16-Jul	17-Jul	18-Jul	03-Aug	04-Aug	05-Aug
55	64	2006/2019	16-Jul	17-Jul	18-Jul	03-Aug	04-Aug	05-Aug
56	65	2006/2019	17-Jun	18-Jun	19-Jun	16-Jul	17-Jul	18-Jul
57	67	2006/2019	23-Jun	24-Jun	25-Jun	22-Jul	23-Jul	24-Jul
58	73	2006/2019	28-Jun	29-Jun	30-Jun	19-Jul	20-Jul	21-Jul
59	74	2006/2019	25-Jul	26-Jul	27-Jul	10-Aug	11-Aug	12-Aug
60	76	2006/2019	22-Jul	23-Jul	24-Jul	10-Aug	11-Aug	12-Aug
61	77	2006/2019	04-Jul	05-Jul	06-Jul	19-Jul	20-Jul	21-Jul
62	78	2006/2019	25-Jul	26-Jul	27-Jul	13-Aug	14-Aug	15-Aug
63	79	2006/2019	25-Jul	26-Jul	27-Jul	13-Aug	14-Aug	15-Aug
64	80	2006/2019	04-Jul	05-Jul	06-Jul	25-Jul	26-Jul	27-Jul
65	81	2006/2019	25-Jul	26-Jul	27-Jul	13-Aug	14-Aug	15-Aug
66	82	2006/2019	04-Jul	05-Jul	06-Jul	13-Aug	14-Aug	15-Aug
67	84	2006/2019	01-Jul	02-Jul	03-Jul	28-Jul	29-Jul	30-Jul
68	86	2006/2019	04-Jul	05-Jul	06-Jul	28-Jul	29-Jul	30-Jul
69	87	2006/2019	04-Jul	05-Jul	06-Jul	28-Jul	29-Jul	30-Jul
70	88	2006/2019	07-Jul	08-Jul	09-Jul	28-Jul	29-Jul	30-Jul
71	89	2006/2019	07-Jul	08-Jul	09-Jul	28-Jul	29-Jul	30-Jul
72	90	2006/2019	07-Jul	08-Jul	09-Jul	31-Jul	01-Aug	02-Aug

73	91	2006/2019	07-Jul	08-Jul	09-Jul	31-Jul	01-Aug	02-Aug

Appendix 2-C. Acoustic sampling schedule of 73 sites across southwest Nova Scotia. Baseline sampling was conducted in 2005/2006 using the Anabat II and resampling in 2018/2019 using the Song Meter SM4BAT-FS. Sites 1-40 were sampled in year one of each data set (2005 and 2018) and 41-91 were sampled in year two (2006 and 2019). Nights selected for resampling and analyses were the first two successful, three-night consecutive sampling periods of the baseline data set between June 5 to August 17. Microphone type used in the resampling data set indicated by the night color where black text indicates a U1 microphone was used while blue text indicates a U2 microphone was used.

Appendix D

Synthetic Playback Test

Appendix 2-D. Playback of a synthetic 40 kHz tone to compare the detection distance of two acoustic monitoring systems used for sampling in southwest Nova Scotia, Canada. Microphones were positioned 10 cm apart and 1.5 m high with the Anabat SD1 centered in a tub with a PVC elbow point and Song Meter SM4BAT-FS with a SMM-U1 and SMM-U2 microphone (Wildlife Acoustics).

Appendix 2-E. Close up of the replicated Anabat acoustic monitoring setup used during 2005/2006 sampling and acoustic equipment comparison tests with an Anabat SD1. The detector and microphone were placed in tub with a PVC elbow joint for weatherproofing.

Appendix E

Long-term Bat Species Detections in Southwest Nova Scotia

Species	Baseline Sampling (2005/2006)	Resampling (2018/2019)
Myotis lucifugus	6,255	10,876
Myotis septentrionalis	72	119
Perimyotis subflavus	349	1,091
Lasiurus cinereus	12	1,488
Lasionycteris noctivagans	29	15
Lasiurus borealis	1	7
Unidentifiable Bat Files	6,216	1,991
Noise Files	25,439	60,520
Total # Files	38,373	76,107

Appendix 2-F. Number of bat detections manually identified to species from acoustic sampling across southwest Nova Scotia, Canada, at 73 forested rivers. Baseline sampling was collected during the summers of 2005/2006 and repeated in 2018/2019. Files that were identified as bat but not to species were grouped as unidentifiable bat files including recordings labelled as species groups and frequency categories. Files not identifiable as bat were labelled a noise.